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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

Based on FACS analysis, intestinal crypt associated CD45+ cells from mice fed with control and high-cysteine diets were sorted into 1.5 ml low-binding tubes with 500 ul FACS buffer. Single cells were processed through the GemCode Single Cell Platform using the GemCode Gel Bead, Chip and Library Kits (10x Genomics, Pleasanton) as per the manufacturer's protocol. The loading cell number is 18,000 for each sample The cells were then partitioned into Gel Beads in Emulsion in the GemCode instrument, where cell lysis and barcoded reverse transcription of RNA occurred, followed by amplification. Amplified cDNA was then used for both 5' gene expression library construction and TCR V(D)J targeted enrichment performed with the Chromium Single Cell V(D)J Enrichment Kit, Mouse T cell (10x Genomics), followed by V(D)J library construction.

Data analysis

The Cell Ranger toolkit (version 3.1.0) provided by 10x Genomics was applied to aggregate raw data, filter low-quality reads, align reads to mouse reference genome (GRCm39), assign cell barcodes, and generate the unique molecular identifier (UMI) matrix. An R-based toolkit, Seurat (version 4.0.1), was used for analyzing the scRNA-seq data. Specifically, the raw UMI matrix was processed to filter out genes detected in less than 10 cells and cells with fewer than 200 genes. We further quantified the numbers of gene and UMI count for each cell, and kept high quality cells with thresholds of 100-2,000 genes and less than 5% mitochondrial gene counts, to ensure that most of the heterogeneous cell types were included for downstream analyses.

Dimension reduction and unsupervised clustering were performed according to the standard workflow in Seurat. Briefly, top 2,000 highly variable genes (HVGs) were detected using the dispersion-based method "vst", where the normalized dispersion is obtained by scaling with the mean and standard deviation of the dispersions for genes falling into a given bin. Principal component analysis (PCA) was performed on the variable gene matrix to reduce noise, and top 15 components were used for downstream analyses followed by "FindNeighbors". Then, "FindClusters" was applied on such nearest neighbor graphs to detect communities and find cell clusters. Of note, the same principal components were also used for non-linear dimension reduction to generate the Uniform Manifold Approximation and Projection (UMAP)

projections for visualization. After the first-round of unsupervised clustering, we annotated each cell cluster according to canonical immune cell markers, and identified the major immune cell types including T and NK cells, B cells, and myeloid cells. We performed a second-round of unsupervised clustering on each major immune cell compartment to obtain the high-resolution map of immune cells in control or CysRD-fed mice. The second-round clustering procedure was the same as the first-round clustering, both of which started from unfiltered expression matrix, and then identified HVGs, calculated PCA matrix, detected cell clusters and performed dimensionality reduction for visualization. All analyses were performed with Seurat. Marker genes were detected using the "FindAllMarkers" function.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Single-cell RNA sequencing dataset generated in this study is available at the Gene Expression Omnibus (GEO) repository (GSE279543). Metabolomics data generated and used for this project are available at Metabolomics Workbench (project ID: PR002592).

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical methods were used to predetermine sample size. Sample sizes were based on those used in previous and preliminary studies from our lab.
Data exclusions	No data were excluded from the analyses.
Replication	The reported data reflects at least two independent experiments, and one representative data is shown. All attempts at replication were successful.
Randomization	Samples (i.e. allocation of sex and age-matched animals to groups) were randomized for all of the experiments.
Blinding	Blinding was not performed in this study due to the low likelihood of author's bias in the final readouts. Analysis of the metabolite data was done in a blinded manner by J.T.H.S.. The authors also followed specific and fixed criteria in analyzing the organoid cultures and histology data according to existing protocols in the lab (used in previous publications).

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

IHC/IF:
IHC-FFPE section: rabbit monoclonal anti-OLFM4 (1:10,000, CST, 39141), rat monoclonal anti-BrdU (1:2000, Abcam, 6326), rabbit polyclonal anti-lysozyme (1:2000, Thermo Fisher, RB-372-A1), rabbit polyclonal anti-RFP (1:500, Rockland, 600-401-379), rabbit polyconal anti-Cleaved Caspase-3 (Asp175) (1:300, CST, 9661), rabbit monoclonal anti-CD8α (1:500, Abcam, ab217344), and rabbit monoclonal anti-HMGCS2 (1:1000, Abcam, ab137043). Biotin-conjugated secondary donkey anti-rabbit IgG (1:500, Jackson ImmunoResearch, #711-066-152), Biotin-conjugated secondary donkey anti-rat IgG (1:500, Jackson ImmunoResearch, #712-066-153). IF-frozen section: rabbit polyclonal anti-EPCAM (1:500, Abcam, ab71916) and rat monoclonal anti-CD8β (1:1000, BioXCell, BE0223).

Immunoblotting (IB):
Rabbit monoclonal anti-HMGCS2 (1:1000, Abcam, ab137043), rabbit polyclonal anti-SLC7A11 (1:1000, Proteintech, 26864-1-AP), rabbit monoclonal anti-phospho-STAT3 (Tyr705) (1:1000, CST, 9145), rabbit monoclonal anti-STAT3 (1:1000, CST, 12640), rabbit monoclonal anti-phospho-S6 Ribosomal Protein (Ser235/236) (1:1000, CST, 4858), monoclonal rabbit anti-S6 Ribosomal Protein (1:1000, CST, 2217), rabbit anti-Cleaved Caspase-3 (Asp175) (1:1000, CST, 9661), rabbit anti-Caspase-3 (1:1000, CST, 9662) and mouse monoclonal anti-Actin (1:10000, Sigma Aldrich, MAB1501).

Flow cytometry:
CD45-APC-Cy7/FITC/PE (BioLegend, 30-F11), CD3-FITC (BioLegend, 17A2), CD4-Pacific Blue (BioLegend, RM4-5), CD8a-PE (BioLegend, QA17A07), CD8b-APC (BioLegend, YTS156.7.7), CD19-BUV737 (BD Biosciences, 1D3), NK1.1-Alexa Flour 700 (BD Biosciences, PK136), NK-1.1-APC (BioLegend, S17016D), TCRγ/δ-APC (BioLegend, GL3), CD127-APC (BioLegend, A7R34), BrdU-PE (BioLegend, 3D4), EPCAM-PE (eBioscience, G8.8). All the FACS antibodies with 1:200 dilution.

In vivo depletion/neutralization:
control IgG (BioXcell, BE0088), anti-CD8β antibody (BioXcell, BE0223); control IgG (BioXcell, BE0091), anti-CXCL10 antibody (BioXcell, BE0440).

Validation

All the antibodies for IHC, Immunoblotting, and flow cytometry are well recognized clones in the field, and validated by the manufacturers. The anti-HMGCS2 (Abcam, ab137043, for IHC, IF, IB) and anti-SLC7A11 (Proteintech, 26864-1-AP, for IB) antibodies were further validated with our tissue specific knockout mouse models.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals

Male and female mice at the age of 8-18 weeks were used in this study. Mice were under the husbandry care of the Department of Comparative Medicine in the Koch Institute for Integrative Cancer Research on a 12h light/12h dark cycle at 20–22 °C with 30–70% relative humidity. All procedures were conducted in accordance with the American Association for Accreditation of Laboratory Animal Care and approved by MIT's Committee on Animal Care.

Mouse Strains:
Lgr5-eGFP-IRES-CreERT2; Rag2-/-; Il22-/-; Il22-Gfp; Lgr5-IRES-CreERT2, Rosa26LSL-tdTomato; Villin-CreERT2, Slc7a11fl/fl; CD8a-Cre, Slc7a11fl/fl; Villin-CreERT2, Hmgcs2fl/fl.

Wild animals

No wild animals were used in this study.

Reporting on sex

Both male and female mice were used in this study.

Field-collected samples

This study doesn't involve the field-collected samples.

Ethics oversight

All animal experiments were in accordance with Committee on Animal Care at MIT.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	The isolated crypts were dissociated to individual cells with TrypLE Express (Invitrogen), 32 °C for 1 min. For crypt associated immune cell isolation, an antibody cocktail comprising (1) CD45-APC-Cy7 (BioLegend, 30-F11), CD3-FITC (BioLegend, 17A2), CD8b-APC (BioLegend, YTS156.7.7), CD19-BUV737 (BD Biosciences, 1D3), NK1.1-Alexa Flour 700 (BD Biosciences, PK136); (2) CD3-FITC (BioLegend, 17A2), CD8a-PE (BioLegend, QA17A07), CD8b-APC (BioLegend, YTS156.7.7); (3) EPCAM-PE (eBioscience, G8.8), CD8b-APC (BioLegend, YTS156.7.7) were used. The antibody staining process was at 4 °C for 20 min in dark. The cells were washed and resuspended in FACS buffer (RPMI 1640 with 2% FBS) with DAPI (Thermo FScientific, 62248; 1:10,000 dilution) or 7AAD (Invitrogen, A1310; 1:500 dilution). For intracellular staining, cells were fixed for 30 min on ice in BD Cytofix/Cytoperm Fixation/Permeabilization (BD Biosciences, 554722) and washed in BD Perm/Wash (BD Biosciences, 554723) diluted 1:10 in ddH2O. Intracellular staining of IL-22-PE (BioLegend, Poly5164) was performed in Perm/Wash buffer for 30 min at 4°C. Cells were washed and resuspended in Perm/Wash buffer.
Instrument	BD FACS Aria
Software	BD FACSDIVA, FlowJo
Cell population abundance	CD45+/Live: 20-30%; CD3+/CD45+: 10-20%; CD19+/CD3-Neg: 60-80%; NK1.1+/CD3-Neg: 2-4%; CD4+/CD3+: 50-70%; CD8a+/CD3+: 60-70%; CD8b+/CD3+: 10-20%; IL22-GFP/CD45+: (IELs: 0.5-1.3%, LPLs: 1.4-1.7%)
Gating strategy	To visualize all populations: FSC-A/SSC-A. For doublet exclusion: FSC-W/FSC-A and SSC-W/SSC-A. To obtain live cells: DAPI or 7-AAD negative population was chosen. For crypt associated immune cells, we selected Epcam-, CD45+ cells. CD3+ cells were then used for CD4+, CD8a+, and CD8b+ sorting, while CD3-Neg cells were used for CD19+ and NK1.1 sorting.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.